Phenotypic detection of virulence determinants and antibiotics resistance in *Staphylococcus aureus* from different clinical isolates in Kirkuk city

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Abstract
Background: Staphylococcus aureus is responsible for hospital and community- acquired infections worldwide. It is capable of causing conditions ranging from minor skin infections to systemic serious diseases such as urinary tract infections (UTIs), pneumonia, osteomyelitis, and endocarditis. Objective: The aim of this study is to isolate and identify S. aureus from different clinical samples and to detect their production of some virulence factors and their correlation with antibiotics resistance. Materials and Methods: A 202 clinical samples were collected from patients of different ages and genders admitted to various hospitals in Kirkuk city (Azadi Teaching Hospital, Kirkuk General Hospital and Public Health Laboratory in Kirkuk city) during the period from November 2021 to June 2022. Staphylococcus aureus were diagnosed based on standard laboratory procedures such cultural characteristics, Gram staining, biochemical tests and VITEK compact 2 system. Virulence factors were also determined using colorimetric agar plates. Antibiotic susceptibility testing was performed by using Kirby-Bauer disc diffusion technique. Methicillin- resistant S. aureus isolates were also identified by oxacillin disc. Result: Out of 202 clinical samples, 65 (32.18%) were positive for S. aureus. These isolates were more predominant among males and in the age group ranges between 21-30 years. S. aureus isolates showed resistance towards benzylpenicillin (92%), oxacillin (68%), (52%) clindamycin, (48%) tetracycline and (48%) cefoxitin. The most effective antibiotics were linezolid, gentamicin and trimethoprim-sulfamethoxazole with a susceptibility rate higher than 84%. Fifty six percent of S. aureus isolates was protease producers, (72%) lipase producer, (76%) and (64%) were lecithinase and capsule producers.
Keywords: *Staphylococcus aureus,* virulence factors, antimicrobial resistant

1. Introduction

Staphylococcus aureus (S. aureus) is a Gram-positive possesses a high degree of versatility and adaptability. It colonizes the mucous membranes and skin of healthy individuals (Shettigar and Murali, 2020). Infections caused by S. aureus are almost caused by strains of the bacteria that have already established a colony in some part of the host's body, turning the host into a reservoir for the further dissemination of the organisms. However, S. aureus can also proliferate in the bloodstream and in various tissues, causing serious diseases (Abdolmaleki et al., 2019). In fact, S. aureus is considered one of the leading causes of hospital and community- acquired infections worldwide. (Darboe et al ., 2019) It is capable of causing conditions ranging from minor skin infections to systemic serious diseases such as urinary tract infections (UTIs), pneumonia, osteomyelitis, and endocarditis (Romero and de Souza, 2021; Raineri et al., 2022; Azmi & Abdeen, 2019). Recurrence, S. aureus occurs in 8–33% of skin, soft-tissue, and bloodstream infections, is a significant aspect of diseases caused by S. aureus. It in turn causes human morbidity and mortality (Neopane et al., 2018). Morbidity and mortality resulting from S. aureus infections can vary greatly depending on clinical entity; the incidence rate can range from 20 to 50 cases/100,000 population per year, which can result in between 10 and 30% fatalities. This incidence rate can vary widely depending on the clinical entity (Khan et al., 2011).

S. aureus causes different aspects of acute infections with the aid of number of virulence factors responsible for these aspects. These factors include enzymes such as coagulase, deoxy ribonuclease (DNase), lipase, proteases (Siddiqui, 2015) and toxins such as enterotoxins, cytolitic toxins, TSST,exfoliative toxins . S. aureus has also capability to form biofilm and capsule. S. aureus produce set of enzymes hydrolyze lipids through destruction of the ester linkage between fatty acids and glycerol, which forms triglycerides. It is thought that this enzyme helps bacteria survival through destruction of host tissues and releasing nutrients (Mahmood, 2021). Proteases produced by S. aureus causes protein catabolism through hydrolyzing the peptide bonds linked amino acids in polypeptides. Staphylococcal proteases are known to cleave and degrade a number of significant host proteins, such as elastin, plasma proteinase inhibitor, and heavy chains of all human immunoglobulin classes. Recent studies have demonstrated that these enzymes are also essential virulence determinants required for S. aureus infections (Barer,2018). Degradation of cell surface proteins such the fibronectin binding protein by

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proteases may also play a role in the transition of S. aureus from an adherent to an invasive condition (Ramrez-Larrota & Eckhard, 2022). S. aureus also secretes number of toxins promote tissue colonization, tissue damage and spread of infections. Among these toxins, hemolysins α, β, γ and δ are commonly produced by S. aureus strains. β-Hemolysin is a sphingomyelinase capable of causing cellular damage in the membranes and significantly affects human immune cell function. Antibiotic resistance is one of Staphylococcus aureus most well-known traits.

The acquisition of determinants by mobile genetic elements via horizontal gene transfer contributed to the evolution of antibiotic resistance to numerous antibiotics in S. aureus (Jensen and Lyon, 2009). Antibiotics are commonly used for treating S. aureus infections, however recently, isolates with diverse resistance have emerged, particularly those that are currently resistant to methicillin and vancomycin therapy (Holmes.,2015). Recent investigations have demonstrated that there are various mechanisms by which S. aureus gained resistance to various antibiotics (Foster, 2017). Therefore, in this study we attempted to detect the prevalence of S. aureus among clinical samples collected from patients and detect their capability to produce virulence factors responsible for S. aureus infections. Antibiotics resistance was also determined.

2. Materials and Methods

Samples collection and bacterial identification

Clinical specimens were collected from patients attended Azadi Teaching General Hospital, Kirkuk General Hospital and Public Health Laboratory in Kirkuk City during the period from November 2021 to June 2022. A total of 202 clinical samples were collected from different clinical specimens which include: urine, blood, swabs (burn, wound, and nostrils). A total of 65 isolates were identified as S. aureus based on cultural features on selective media, Gram staining, biochemical tests such as coagulase, catalase, oxidase and DNase test and confirmed by using VITEK.

Detection of S. aureus virulence determinants

1.Protease assay

This medium was prepared by dissolving 51.5 g of milk agar in 1000 ml of distilled water, and boiling until the media was completely dissolved. This media was autoclaved at 121˚C for 15 minutes, and left to cool down (45-50℃) (Collee et al., 1996). The media was then poured into sterile petri dishes. Bacteria producing protease appeared as a clear zone surrounded inoculated bacteria.

2.Lecithinase and lipase tests

It was prepared by adding 15 ml of Egg-yolk suspension to 85 ml of pre-prepared sterile nutrient agar and poured into sterile plates. Bacteria that produced lecithinase appeared as halo zone of insoluble precipitate (Goldman and Green, 2015). Lipase enzyme was also detected on the same plate by immersing the plate with adequate amount of 20% (CuSO4. 7H2O) for 20 minutes. The excess solution was removed from inoculated plate and incubated at 37°C for 30 minutes to dry. The appearance of bluish green color in the areas of growth indicated a positive result (Koneman et al., 2006).

3.capsule detection

Capsule was detected by using negative staining dye (Indian ink) by placing a drop of Indian ink on a sterile glass slide and mixed with overnight bacterial culture growing on Brain heart infusion agar and covered with cover slip. The slide was then examined under light microscope. The presence of transparent, non-pigmented halo around the bacterial cell indicated the presence of capsule (McPherson, 2017).

4. Hemolytic assay

This test was used to determine the capability of S. aureus to produce hemolysins. The examined bacteria were cultured on blood agar and incubated overnight in Candle Jar at 37°C. The formation of a clear-zone around the bacterial colony indicated presence of beta hemolysin (beta hemolysis), while appearance of green color indicated the production of alpha hemolysin (alpha hemolysis) and no changes in blood agar indicated the presence of gamma hemolysin (γ-hemolysis) (Baron et al.,1994).

Antibiotic susceptibility test

Vitek 2 AST compact was used to detect S. aureus susceptibility towards selected antibiotics. Kirby-Bauer disc diffusion method was used for detection bacterial resistance towards cefoxitin disc. This test was conducted by preparation of bacterial suspension and compared with 0.5 McFarland standard tube (1.5x 108 CFU/ml). This suspension was then inoculated into Muller Hinton agar and Cefoxitin (30 µg) disc was placed. The inoculated plate incubated overnight at 37°C. The diameter of inhibition zone around the disc was recorded and interpretated in accordance with Clinical Laboratory Standard Institute (CLSI) guideline (CLSI, 2022). The isolates were considered resistant if inhibition zones ≤ 25 and sensitive if inhibition zones ≥ 24 as shown in Appendix (2-A). In Vitek antibiotic sensitivity test, minimal inhibitory concentration (MIC) was used to detect multi drug resistant S. aureus in which the isolates resistant to at least one in three or more antimicrobial classes (Appendix 2 -B).

3. Results

Isolation and identification of *S. aureus*

A total of 202 samples were collected from patients attended Kirkuk hospitals from the period of November 2021 to June 2022. The samples were collected from different clinical sources (blood, urine, nasal swabs, wound and burns), and from different ages and genders. The results of current study showed that 65 (32.21%) of clinical isolates was

Staphylococcus aureus which were confirmed based on the colony morphology, Gram staining and biochemical tests. Growth on Mannitol salt agar, catalase, coagulase and DNase tests are considered important phenotypic identification markers for S. aureus. All S. aureus colonies in this study appeared as smooth and yellow-pigmented on mannitol salt agar due to mannitol fermentation and acid production as seen in Figure (1-A). Under light microscope, S. aureus cells appeared as grape-like clusters with purple color (Gram positive) as seen in Figure (1-B). All S. aureus isolates were DNase positive by appearance of clear halo zone surrounding the inoculated colonies as seen in Figure (1-C), (100%) positive for the coagulase test (slide and tube methods) as seen in Figure (1- E, F). and catalase test by appearing of air bubbles after the addition of H2O2 solution. as seen in Figure (1-D). On the other hand, all S. aureus isolates were negative for oxidase test as shown in Figure (1-G). S. aureus isolates was further confirmed by using VITEK 2 compact system. This system is rapid, accurate and can be easily used for identification of suspected isolates to the species level. The results of VITEK-2 were identical to those obtained by conventional laboratory methods as provided in Appendix (2-A).

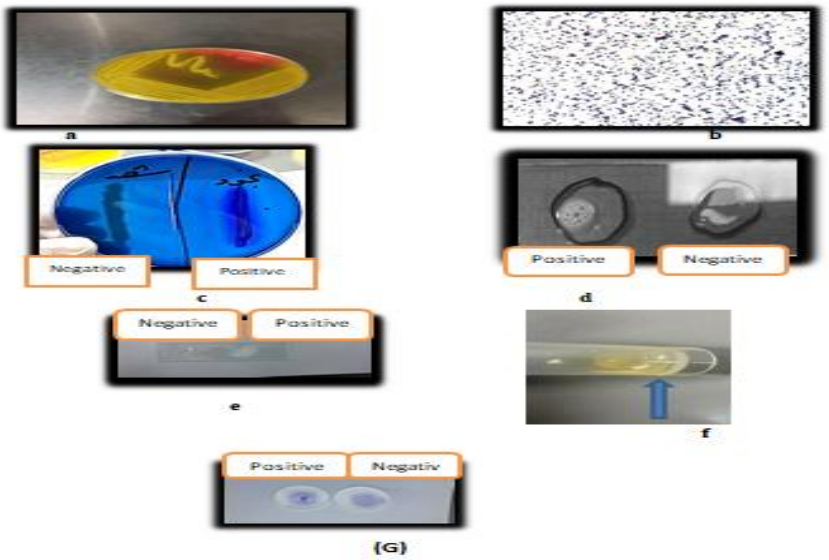


Figure 1: Shows identification profile of Staphylococcus aureus. (A): S. aureus growing on selective media Mannitol salt agar, (B): S. aureus cells stained with Gram and visualized under light microscope, (C): DNase test on DNA agar, (D): Catalase test by using 3% of H2O2, (E): Coagulase slide test (Clumping factor), (F): Tube coagulase test (Free coagulase enzyme) using human plasma and (G): Oxidase test by using tetramethyl—phenylene-diamine dihydrochloride.

Prevalence of *S. aureus* isolated from the different clinical samples

Among 65 (32.18%) positive S. aureus isolates, 10 (15.39%) was discovered from blood samples, 17 (26.15%) from urine, 12 (18.46%), 6 (9.23 %) and 20 (30.77%) were isolated from wounds, burns and nostrils as exhibited in Table (1).

Table 1: Prevalence of S. aureus isolated from different clinical samples

Source	Total	Positive	Negative
		No (%)	No (%)
Blood	30	10 (15.39)	20 (14.59)
Wound	60	12 (18.46)	48 (35.04)
Urine	67	17 (26.15)	50 (36.50)
Burns	20	6 (9.23)	14 (10.22)
Nasopharynx	25	20 (30.77)	5 (3.65)
Total	202	65 (32.18)	137 (67.82)

According to the results of current study, the percentage of nasal swabs 20 (30.77%) was found to be considerably higher than that in other clinical samples (blood, burns, urine, and wounds). Furthermore, 20 (80%) was isolated from inpatients and 5 (20%) from outpatients. The results also found that 55.38% of patients were males and 44.62% were females, and 38.46% of patients were belonged to the age group 21-30 year and 27.69% were within the age group 11-20 year as shown in Table (2).

Table 2: Distribution of S. aureus isolates according to the age groups and sex.

Age groups (years)	No.	%
1-10	11	16.92
11-20	18	27.69
21-30	25	38.46
31-45	11	16.92
Total	65	100
Sex		
Males	36	55.38
Females	29	44.62
Total	65	100

Antibiotic sensitivity test

Antimicrobial susceptibility pattern of S. aureus isolates was examined against 16 different antibiotics using disc diffusion method and VITEK 2 susceptibility test. The presence of clear zone around S. aureus colonies in disc diffusion method indicates the sensitivity of bacterial isolates to selected antibiotic (cefoxitin) as shown in Appendix (1). While minimal inhibitory concentrations (MICs) were used

to detect susceptibility of selected antibiotics as shown in Appendix (2-A). Multidrug-resistant S. aureus infections are a significant issue, due to difficulty for their treatment. In this work, multiple resistance profile was observed among S. aureus isolates. They showed a high rate of resistance towards penicillin and its derivatives [(92%) was resistant to Benzyl penicillin, (68%) oxacillin (Methicillin) as well as second generation of cephalosporin (Cefoxitin 48%)]. On the other hand, all S. aureus isolates showed susceptibility to tigecycline (100%), 92% linezolid, 88% sensitive to (gentamicin, teicoplanin, vancomycin), 84% rifampicin and Trimethoprim/ Sulfamethoxazole, 72% sensitive to fusidic acid, and 68 % sensitive to Moxifloxacin and Erythromycin (Figure 2).

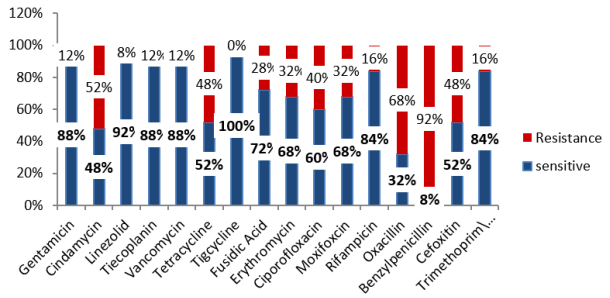


Figure 2: Antibiotic sensitivity of Staphylococcus aureus.

Detection of Virulence factors

Staphylococcal extracellular enzymes such as protease, lipase, hemolysin and lecithinase as well as capsule production were detected in 25 multi-resistant S. aureus isolates.

Hemolytic activity

Based on hemolytic activity on blood agar plates (Figure 3), the results showed that all selected S. aureus isolates formed a typical β hemolytic phenotype on blood agar plate by appearance of a clear zone surrounded the colonies and it is referred as beta hemolysis.

Figure 3: S. aureus on blood agar plate

Capsule production

Indian ink was used for staining of capsule present in the clinical isolates. Appearance of transparent, non-pigmented halo around the bacterial cell indicated the presence of capsule (Figure 4). The results showed that 16 out of 25 (64%) of S. aureus isolates were capsule producers (Table 3).

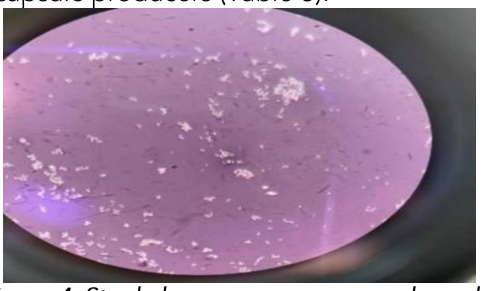


Figure 4: Staphylococcus aureus capsule under microscope

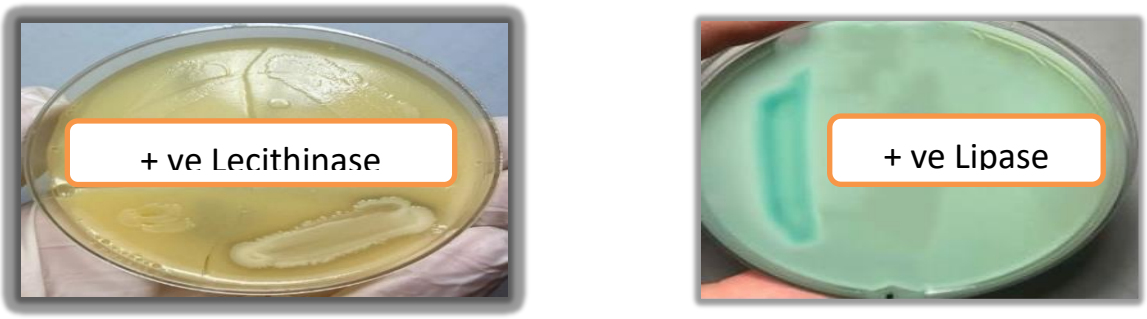
3. Protease activity

S. aureus producing protease appeared as glistening orange convex colonies on skim milk agar due to Staphyloxanthin production with a clear zone surrounding the colonies (Figure 5) and 56% of S. aureus isolates were protease positive (Figure 5 and Table 3).

Figure 5: S. aureus growing on skim milk agar

Lecithinase and lipase activity

Positive lipase strains appeared as a clear zone surround the inoculated colony, while lecithinase producer strains appeared as a zone of insoluble precipitated (Figure 6). The results also showed that 72% of *S. aureus* isolates produced lipase enzyme on Egg Yolk agar after addition of aqueous copper sulfate, and 76% of isolates have lecithinase activity (Table 3).



(B)

Figure 6: (A)- Lecithinase activity of S. aureus on Egg Yolk agar. (B)- Lipase activity of S. aureus on Egg Yolk agar after addition of $CuSO_4$-$7H_2O$.

Table 2: Extracellular enzymes of S. aureus			
Enzymes	Positive (%)	Negative (%)	Total (%)
Capsule	16 (64)	9 (36%)	25 (100)
Hemolysin	25 (100)	0(0)	25 (100)
Lipase	18 (72)	7 (28)	25 (100)
Protease	14 (56)	11 (44)	25 (100)
Lecithinase	19 (76)	6 (24)	25 (100)

4. Discussion

S. aureus is a major human pathogen that causes a diverse range of hospital and community acquired infections (Zhao et al., 2018). All S. aureus isolates in this study showed 100% positive for DNase. This finding is in agreement with other studies. Shakir and Mohammad Ali, (2014) in Kirkuk city found that all S. aureus isolated from women with urinary tract infections were DNase producers. However, the work performed by (Türkyilmaz and Kaya, 2006) reported that the positive rate for DNase test among positive coagulase staphylococci was only 42.2%. and the results also showed that all S. aureus isolates were positive (100%) for the coagulase test by using two methods (slide and tube methods). This result was consistent with the findings of Shakir and Mohammed Ali (2014) work done in Kirkuk city, who found that 100% of S. aureus isolates in women with UTI was coagulase positive. The percentage of nasal swabs 20 (30.77%) was found to be considerably higher than that in other clinical samples, similar to other researches (AL-Kazaz, 2014). Ahluwalia et al (2000). This result was also supported by earlier studies (Hussein, 2009; Zidane, 2007). Conversely, Hatem (2017) showed the lowest frequency and distribution of S. aureus from nasal swabs. As indicated in Table (1), the lowest isolated rate was recorded in burn infections 6 (9.23%). This result is lower than that recorded in the study of (Jowad, 2013) who found that (18.1%) of burn swabs was staphylococcus aureus. While, this finding disagrees with (Eliasen, 1997) results. The 26.15% of S. aureus was isolated from urine. This rate is higher than that reported by Al-Muaini (2006) and lower than (Derakhshan et al., 2021) findings 49.6%. Kandel et al. (2020) showed high prevalence of S. aureus among localized wound infections (41.1%), which is higher than that reported in the present study (18.46%). In a study done by Derakhshan et al. (2021) revealed that 17.1% of clinical S. aureus strains was isolated from wounds, consistent with our results. Finally, S. aureus was isolated from 15.39% of blood cultures, which is comparable to the findings of previous studies (Derakhshan et al., 2021). The current study was exhibited 20 (80%) isolates from inpatients and 5 (20%) from outpatients. Close to our results, Derakhshan et al. (2021) reported a total of 97 strains (78.9%) were isolated from inpatients, and 26 (21.1%) from outpatients. Azizi (2013) found that the rate of isolated Staphylococcus aureus bacteria was (19.23%). Our results were similar to the results found by Alshamari (2014). These variations could be attributed to differences in sample sizes, laboratory techniques and geographic variation.

The results of present study also found that 55.38% of patients were males and 44.62% were females (Table 2), our finding agreed with the study done by Al- Zaidi, (2014) whom found that males represented 56.7% of infected patients and 43.3% was females. Conversely, Saeed et al., (2014) found that 44.5% of S. aureus isolates were males and 55.5% were females. Derakhshan et al., (2021) also revealed that males are more vulnerable to S. aureus infections than females. Another research reported that 56.4% of S. aureus infections were in males and 43.6% in females. This discrepancy is more likely due to variation in behavioral and physiological factors such as hand hygiene and sex hormone especially androgen which stimulates Staphylococcal infections (Kandel et al., 2020). The current study also showed that 38.46% of patients belonged to age group 21-30 year and 27.69% were within the age group 11-20 year as shown in Table (2). These findings were comparable to the study performed by Mohammed (2012) who stated that S. aureus was carried by the majority of young people due to the hormonal fluctuation in young people being enhances the risk of acquiring S. aureus in those groups. In addition, Al-Zaidi, (2014), found that S. aureus infections are more frequently in the age group 20–29 years. Chen et al., (2017) also found that the higher rate of nasal carriage was in people under the age of 20 years, and Yan et al., (2015) mentioned that people under the age of 24 have the highest likelihood of S. aureus colonization. The pathogenicity of S. aureus was attributed to different virulence factors, including a large number of cell-surface-bound proteins expressed during host colonization, and proteins such as hemolysins, proteases, and lipases secreted during acute infections (Afzal et al., 2022). These factors also enhance Staphylococcal capability to resist antibiotics and confront natural host defenses, which subsequently aggravate the infection outcomes (Alsamarai et al., 2015). Regarding lipase and lecithinase activity, the results showed that 72% of S. aureus isolates produced lipase enzyme on Egg Yolk agar after addition of aqueous copper sulfate, and 76% of isolates have lecithinase activity. These results are in agreement with previous study demonstrated that 71.4% of S. aureus isolates produce lipase enzymes (AlDouri, 2009). Research performed by Shakir and Mohammed Ali (2014) exhibited 76.47% of lipase production. These findings are also comparable to those recorded by Salman and Ali, 2016, who revealed that 80% of isolates were able to produce this enzyme. Similar result was obtained by El-baz and his group in 2016 found that 81% of isolates presented lipolytic activity. Abdul-Kreem and Husain in 2015 reported that all S. aureus isolates were able to produce lipase. Al-Shammary and Al-Husseiny in 2014 reported that lipase could be used by S. aureus in a variety of ways to establish the infection in various body sites. Recent study reported that lipase enzyme plays a supreme role in the severity of infection as it

has capability to hydrolyze triglycerols for nutrient acquisition (El-baz et al., 2016). It was also reported that lipase enzyme is important for formation of abscesses and organs invasion as high counts of bacteria (104 -107 CFU/g) was observed in the kidneys, spleens and livers of rats inoculated with S. aureus (dos Santos Rodringues et al., 2014). Furthermore, lecithinase enzyme is important for breaking down phosphorous lipids in cell membranes, causing tissue decomposition and disintegration (Sharaf et al., 2014). In agreement with our results, Shakir and Mohammad Ali (2014) in Kirkuk, Iraq found that 82.35% of S. aureus isolates from women with UTI were lecithinase positive. This rate was relatively higher than those reported by AlDouri, 2009, who reported a percentage of 59.1% of isolates was lecithinase positive. on the other hand, research done by Abdul-Kreem and Husain (2015) showed that all S. aureus isolates 100% were capable to produce lecithinaes. Another virulence determinant is the production of protease enzymes. S. aureus produce these enzymes appeared as glistening orange convex colonies on Skim milk agar due to Staphyloxanthin production with a clear zone surrounding the colonies. Staphylococcal proteases could cleave and degrade a number of important host proteins causing destruction of tissues (Karlsson & Arvidson, 2002). In the present study, 56% of S. aureus isolates had protease activity. In agreement with earlier results, Bahaa EL Din, (2018) in Kirkuk, Iraq, found that 48% of S. aureus isolates produce protease enzyme among selected clinical samples. Shakir and Mohammad Ali (2014) found that 29.41% S. aureus isolates were able to produce protease, which is relatively lower than that reported in our study. On the other hand, Salman and Ali (2016) and Hatem (2017) found that 85% and 70% of S. aureus isolates had the ability to produce this enzyme respectively, which are higher than that reported in the current study.

The results also showed that all selected S. aureus isolates formed a typical β hemolytic phenotype on blood agar plate. These results are similar to those reported by Al-kazaz in 2014 (95.4%), while this percent is higher than that recorded by Al Ani and Al Meani in 2018 who found that 65.6% of S. aureus isolates caused beta hemolysis on blood agar plate.

The results also showed that 16 out of 25 (64%) of S. aureus isolates produced capsule, similar to Shakir and Mohammed Ali (2014) report in Kirkuk, who discovered that S. aureus isolated from UTI infected women can produce capsule, which is consistent with our findings. Türkyilmaz and Kaya (2006) also reported that 53.3% of isolates were capsule procedures. This number however is lower than that reported by Luong et al., 2002 who reported that 90% of S. aureus were capsule producers.

Antibiotic sensitivity results are consistent with previous studies showed high prevalence of resistance towards methicillin among S. aureus clinical isolates and healthy carriers (Romero et al., 2021 and Al Zaidi, 2014). However, Abulreesh et al. (2017) revealed low number of MRSA isolates among different clinical samples in Saudi Arabia. This variation among different regions in the same country and across countries might be due to differences in geographical regions and health system and control program (Abulreesh et al., 2017). Resistance to ciprofloxacin, tetracycline, and clindamycin was revealed by (40%), (48%), and (52%) of the isolates, lower than that reported by Abulreesh et al. (2017). Furthermore, our results were similar to Derakhshan (2021) study who found that (94.3%) of S. aureus strains were sensitive to gentamicin, (97.6%) trimethoprim-sulfamethoxazole and 100% linezolid. Vancomycin sensitivity result in this study is in agreement with the results of other researchers (Rania et al., 2015) in which S. aureus isolates were sensitive to vancomycin (87.5%). In Younus and ŞİMŞEK, (2022) study, S. aureus showed high rate of sensitivity towards gentamycin, Rifampicin, with 100% resistant to amoxicillin, oxacillin and tetracycline. Mama et al. (2019) found that S. aureus isolates were resistant to amoxicillin (100%), ampicillin (86.4%), amikacin (8.1%), erythromycin (54%), tetracycline (54%), cefotaxime (54%), methicillin (21.6%), and vancomycin (10.8%). Staphylococcus aureus might gain resistance to various antibiotics by using different ways such as antibiotics inactivation, modification of antibiotic targets or changes in the permeability of cell membrane and developing of drug efflux system. Resistant plasmids also play an important role in the transfer of resistance genes through transformation, conjugation, transduction and other mechanisms (Gatadi et al., 2019).

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References

Abdolmaleki, Z., Mashak, Z., & Safarpoor Dehkordi, F. (2019). Phenotypic and genotypic characterization of antibiotic resistance in the methicillinresistant Staphylococcus aureus strains isolated from hospital cockroaches. Antimicrobial Resistance & Infection Control, 8(1), 114

AbdulKareem, H. S., & Husain, A. S. (2015). Genetic Comparative Study of Staphylococcus aureus& Staphylococcus epidermidis Isolated from Wounds, Burns and Skin Flora. Iraqi Journal of Science, 56(1), 708712

Abulreesh, H. H., Organji, S. R., Osman, G. E., Elbanna, K., Almalki, M. H., & Ahmad, I. (2017). Prevalence of antibiotic resistance and virulence factors encoding genes in clinical Staphylococcus aureus isolates in Saudi Arabia. Clinical epidemiology and global health, 5(4), 196202

Afzal, M., Vijay, A. K., Stapleton, F., & Willcox, M. (2022). Virulence Genes of Staphylococcus aureus Associated with Keratitis, Conjunctivitis, and Contact

Lens–Associated Inflammation. Translational vision science & technology, 11(7), 55.
Ahluwalia, A., Sood, A., Sood, A., Lakshmy, R., Kapil, A. and Pandey R. M. (2000). Nasal colonization with Staphylococcus aureus in patients with diabetes mellitus. Diabetic Medicine, 17 (6): 487-488
Al Ani, ATA and Al Meani, SAA (2018). Molecular Screening of Adhesion Proteins Genes in Staphylococcus aureus Strains Isolated from Different Clinical 104 Infections in Baghdad City and Identification of Their Relationship with Some Virulence Factors. Journal of AlNahrain University; 21(1): 7989
AlDouri, Mohammed Natir known.(2009) Genetic study of some bacteria isolated from lower tract infections in adults in Tamweet, PhD thesis, College of Education, University of Tikrit
ALKazaz, E. J. N. (2014). Biochemical and Molecular Study of Staphyloxanthin Extracted from Clinical Isolates of Staphylococcus aureus. M.Sc. Thesis, College of Science, University of Baghdad, Baghdad
Alsamarai, A. M., Abbas, H. M., & Atia, Q. M. (2015). Nasal carriage of methicillin resistant Staph aureus in food provider in restaurant at Samara city. World Journal of Pharmacy and Pharmaceutical Sciences, 4(6), 5058.
AlShammary, AHR and AlHusseiny, AFR (2014). Extraction, Purification and Characterization of Lipase Produced by a Local Isolate of Staphylococcus aureus. Iraqi Journal of medical sciences; 12(3): 254260.
AlUgaili, D. (2013). Bacteriological and Genetic Studies on Oxacillin Resistant Staphylococcus aureus Isolated from Some Hospital in Baghdad City (Doctoral dissertation, Ministry of Higher Education
AlZaidi, JR (2014). Methicillin Resistant Staphylococcus Aureus(MRSA) Nasal Carriage among Health Care Workers in Intensive Care Units. Medical Journal of Babylon; 11(3(: 74975
Aziz, S.A. (2013). Bacterial urinary tract infection in diabetic patients in AlNajaf city. M.Sc. Thesis,Collage of Medicine, University of Kufa, Iraq
Azmi, K., Qrei, W., & Abdeen, Z. (2019). Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of Staphylococcus aureus isolated from Palestinian patients. BMC genomics, 20(1), 112.
Baha AlDin Alaa Amir (2018). Investigation for the gene encoding pvl and some virulence factors in Oxacillinresistant Staphylococcus aureus bacteria isolated from different clinical and environmental sources in Kirkuk Governorate, University of Kirkuk
Barer, Mike, William L. Irving, Andrew Swann, and Nelun Perera. 2018.Medical microbiology: a guide to microbial infections : pathogenesis, immunity, laboratory investigation and control. https://www.clinicalkey.com/#!/browse/book/3-s2.0-C20150065212.
Baron, E. J.; Peterson, L. R. and Finegold, S. M. (1994). Bailey and Scott's Diagnostic Microbiology. 9th Ed. The C.V. Mosby Company, U.S.A
Chen, BJ; Xie, XY; Ni, LJ et al. (2017). Factors associated with Staphylococcus aureus nasal carriage and molecular characteristics among the general population at a Medical College Campus inconsumer protection; Guangzhou, South China. Annals Clinical Microbiology Antimicrobials; 16(28): 110
Collee, J. G., Miles, R. S. & Watt, B. (1996). Tests for identification of bacteria. Mackie & McCartney, practical medical microbiology, Churchil Livingston, 14, 131149.
Darboe, S., Dobreniecki, S., Jarju, S., Jallow, M., Mohammed, N. I., Wathuo, M., ... & Secka, O. (2019). Prevalence of Panton-Valentine leukocidin (PVL) and antimicrobial resistance in community-acquired clinical Staphylococcus aureus in an urban Gambian hospital: a 11-year period retrospective pilot study. Frontiers in cellular and infection microbiology, 9, 170.
Derakhshan, S., Navidinia, M., & Haghi, F. (2021). Antibiotic susceptibility of humanassociated Staphylococcus aureus and its relation to agr typing, virulence genes, and biofilm formation. BMC infectious diseases, 21(1), 110.
Divyakolu, S., Chikkala, R., Ratnakar, K. S., & Sritharan, V. (2019). Hemolysins of Staphylococcus aureus—An update on their biology, role in pathogenesis and as targets for antivirulence therapy. Advances in Infectious Diseases, 9(2), 80104.
dos Santos Rodrigues, J. B., Pinto, T. S., de Oliveira, C. P., de Sousa Freitas, F. I., Pereira, M. D. S. V., de Souza, E. L., & de SiqueriaJúnior, J. P. (2014). Lipolytic activity of Staphylococcus aureus from human wounds, animals, foods, and foodcontact surfaces in Brazil. The Journal of Infection in Developing Countries, 8(08), 10551058.
Elbaz, R., Rizk, D.E., Barwa, R., Hassan, R. (2016). Virulence factors profile of Staphylococcus aureus isolated from different clinical sources. J. Microbiol. 43, 126144.
Elbaz, R., Rizk, D.E., Barwa, R., Hassan, R. (2016). Virulence factors profile of Staphylococcus aureus isolated from different clinical sources. J. Microbiol. 43, 126144.
Foster, T. J. (2017). The Staphylococcus aureus "superbug". The Journal of clinical investigation, 114(12), 16931696
Gatadi, S., Lakshmi, T. V., & Nanduri, S. (2019). 4 (3H)-Quinazolinone derivatives: Promising antibacterial drug leads. European journal of medicinal chemistry, 170, 157-172.
Goldman, E., & Green, L. H. (2015). Practical handbook of microbiology. 4th Ed. CRC press
Hatem, ZA (2017). Investigation of Virulence Factors of Staphylococcus. aureus That Isolated from Different Clinical Infections in Baladroz City (Iraq). Dyala Journal for pure science; 13(3): 16417
Holmes, N. E., Tong, S. Y., Davis, J. S., & Van Hal, S. J. (2015, February). Treatment of methicillin-resistant Staphylococcus aureus: vancomycin and beyond. In Seminars in respiratory and critical care medicine (Vol. 36, No. 01, pp. 017-030). Thieme Medical Publishers.
Hussein Ahmed Hamed Mahdi Shukran 2009 Isolation and identification of staphylococcus and streptococcus bacteria from upper respiratory tract infections and their sensitivity to antibiotics, Master's

Thesis, College of Education, Tikrit University
Jensen, S. O., & Lyon, B. R. (2009). Genetics of antimicrobial resistance in Staphylococcus aureus. Future microbiology, 4(5), 565582
Jevon, M., Guo, C., Ma, B. Mechanisms of internalization of Staphylococcus aureus by cultured human osteoblasts. Infection and Immunity 1999; 67 (5): 2677-2681
Jowad. Mustafa Raad .(2013).Detection of some genes for virulence factorsAntibiotic resistance in staphylococcal bacteriaIsolated from clinical cases different in Diwani city . Master's Thesis College of AlQadisiya UniversityIraq
Kandel, S. N., Adhikari, N., Dhungel, B., Shrestha, U. T., Angbuhang, K. B., Karki, G., ... & Ghimire, P. (2020). Characteristics of Staphylococcus aureus isolated from clinical specimens in a tertiary care hospital, Kathmandu, Nepal. Microbiology Insights, 13, 1178636120972695
Karlsson, A., Arvidson, S. (2002) Variation in extracellular protease production among clinical isolates of Staphylococcus aureus due to different levels of expression of the protease repressor sarA. Infection and Immunity, 70(8), 42394246.
Khan, F., Shukla, I., Rizvi, M., Mansoor, T., & Sharma, S. C. (2011). Detection of biofilm formation in Staphylococcus aureus. Does it have a role in treatment of MRSA infections. Trends Med Res, 6(2), 11623.
Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schreckenberger, P.C. and Winn, W.C.(2006). Color atlas and textbook of diagnostic microbiology.6th.ed
Lauková, L., Konečná, B., Janovičová, Ľ., Vlková, B., & Celec, P. (2020). Deoxyribonucleases and their applications in biomedicine. Biomolecules, 10(7), 1036.
Luong, T., Sau, S., Gomez, M., Lee, J. C., & Lee, C. Y. (2002). Regulation of Staphylococcus aureus capsular polysaccharide expression by agr and sarA. Infection and immunity, 70(2), 444450.
Mahmood, A. P. S. S. (2021). Hashim Yousif Yassin (Doctoral dissertation, University of Baghdad).
McPherson, R. A. (2017). Henry's Clinical Diagnosis and Management by Laboratory Methods: First South Asia Edition_eBook. 7th Ed. Elsevier India.
Mama, M., Aklilu, A., Misgna, K., Tadesse, M., & Alemayehu, E. (2019). Methicillin-and Inducible Clindamycin-Resistant Staphylococcus aureus among Patients with Wound Infection Attending Arba Minch Hospital, South Ethiopia. International journal of microbiology, 2019.
Neopane, P., Nepal, H. P., Shrestha, R., Uehara, O., & Abiko, Y. (2018). In vitro biofilm formation by Staphylococcus aureus isolated from wounds of hospitaladmitted patients and their association with antimicrobial resistance. International journal of general medicine, 11, 25
Omae Y, Sekimizu K, Kaito C (2014) Identification of Staphylococcus aureus 123 Colony-Spreading Stimulatory Factors from Mammalian Serum. PLoS ONE 9(5):e97670 from Mammalian Serum. PLoS ONE 9(5): e97670
Prokešová, L., Potužníková, B., Potempa, J., Zikán, J., Radl, J., Hachová, L., ... & John, C. (1992). Cleavage of human immunoglobulins by serine proteinase from Staphylococcus aureus. Immunology letters, 31(3), 259265.
Raineri, E. J., Altulea, D., & van Dijl, J. M. (2022). Staphylococcal trafficking and infection—from 'nose to gut'and back. FEMS Microbiology Reviews, 46(1), fuab041
Rania Talaat, A. H., Magda Mohammad, E. N., Nesreen Salah Omar, O., Mamdouh Radwan El Nahas, E. N., & Rawia Ibrahim, B. (2015). Identification of virulence factors, accessory gene regulator [agr] locus and antibiotic resistance pattern of Staphylococcus aureus isolated from diabetic foot ulcer
Romero, L. C., & de Souza, M. D. L. R. (2021). Insights into the epidemiology of communityassociated methicillinresistant Staphylococcus
Salman, AD and Ali, EA (2016). Identification Of Staphylococcus Aureus Isolated From Different Infections And Study The Ability Of Nuclease Production. Dyala Journal for pure science; 13(2): 215225.
Shakir, N. B., & Mohammed Ali, Kh. O., (2014). Bacteriological Study of Urinary Tract Infections in Married Women and Evaluation of Some Virulence Factors of Staphylococcus aureus Master thesis, College of Medicine, Tikrit University.
Shettigar, K., & Murali, T. S. (2020). Virulence factors and clonal diversity of Staphylococcus aureus in colonization and wound infection with emphasis on diabetic foot infection. European Journal of Clinical Microbiology & Infectious Diseases, 39(12), 22352246
Shetty, N., Tang, J.W., Andrews, J. (2009) "Infectious Disease: Pathogenesis, Prevention and Case Studies". John Wiley & Sons.
Saeed, EN; AlDeen, HIO and Merdaw, MA (2014). Determine Nasal Carriage of Methicillin Resistant Staphylococcus aureus MRSA in Young Adult College Student. Iraqi Journal of Pharmaceutical Science; 23(1): 8388
Siddiqui, Y. (2015). Phenotypic Assessment of Exoenzyme Activity by Clinical Isolates of Staphylococcus aureus. RADS Journal of Biological Research & Applied Sciences, 6(1), 131
Türkyilmaz, S., & Kaya, O. (2006). Determination of some virulence factors in Staphylococcus spp. isolated from various clinical samples. Turkish Journal of Veterinary & Animal Sciences, 30(1), 127132
Younus, F. & ŞİMŞEK, S., A., (2022). Molecular identification of staphylococcus aureus strains isolated from the respiratory paths of Iraqi patients. Master Thesis, Department of Biology, Çankırı Karatekin Universit
Yan, X; Song, Y; Yu, X et al. (2015). Factors associated with Staphylococcus aureus nasal carriage among healthy people inNorthern China. Clinical Microbiology and Infection; 21(2):
Zhao, A. (2018). Studies of the Biosynthesis, Signaling, and Manipulation of the Staphylococcal Agr Quorum Sensing Circuit (Doctoral dissertation, Princeton University

Appendix

Appendix 1

bioMérieux Customer: Microbiology Chart Report Printed March 21, 2022 11:50:48 AM AST

Patient Name: Patient ID:
Location: Physician:
Lab ID: 187 N Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

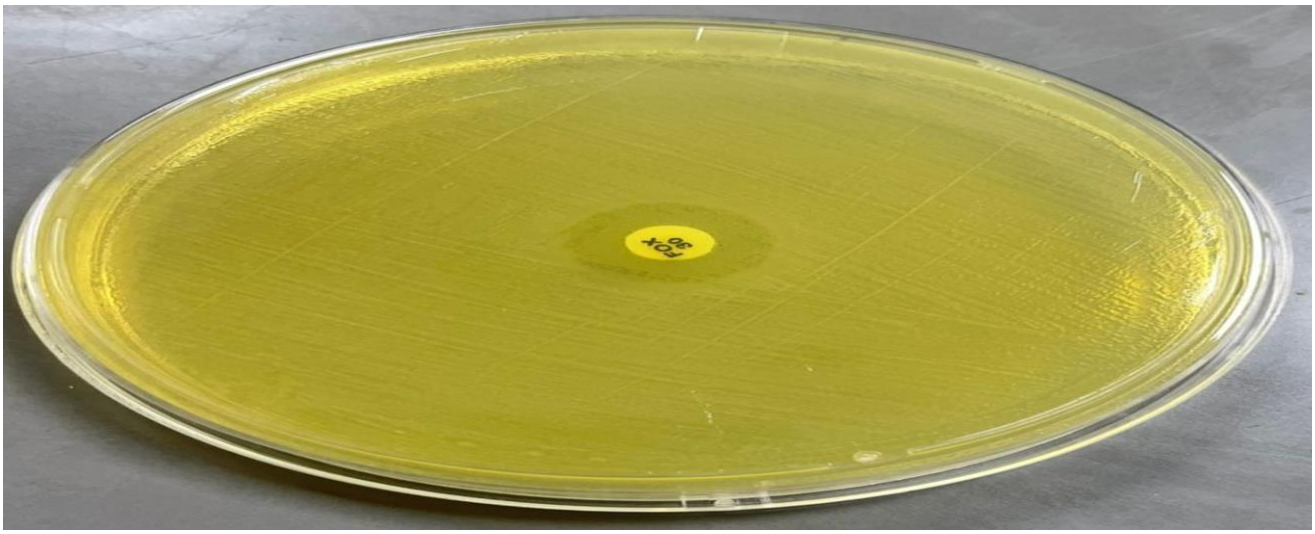
Comments:	

Identification Information	**Analysis Time:** 4.78 hours	**Status:** Final
Selected Organism	99% Probability **Staphylococcus aureus** **Bionumber:** 050402065361231	
ID Analysis Messages		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix 2-A



Appendix 2-B

bioMérieux Customer: Microbiology Chart Report Printed February 23, 2022 10:47:23 AM AST

Patient Name: Patient ID:
Location: Physician:
Lab ID: 193 N Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 13.85 hours	**Status:** Final

Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Benzylpenicillin	>= 0.5	R	Vancomycin	>= 32	R
Oxacillin	<= 0.25*	*R	Tetracycline	>= 16	R
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Ciprofloxacin	>= 8	R	Fosfomycin	<= 8	S
Moxifloxacin	2	R	Fusidic Acid	2	R
Clindamycin	>= 8	R	Rifampicin	>= 32	R
Linezolid	>= 8	R	Trimethoprim/ Sulfamethoxazole	<= 10	S
Teicoplanin	>= 32	R			

***= AES modified **= User modified**

AES Findings	
Confidence:	Consistent with correction